A Novel Mutation of a Leucine Residue in Coil 1A of Keratin 9 in Epidermolytic Palmoplantar Keratoderma

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Keratin 9 mutation was examined in a Japanese kindred of epidermolytic palmoplantar keratoderma (EPPK), which is a dominantly inherited autosomal disorder of keratinization characterized by diffuse thickening of the palms and soles and by epidermolytic hyperkeratosis histologically. We report herein a novel mutation, a C → G transversion at nucleotide position 541 that converts a leucine residue (CTC) to a valine (GTC) at codon 159. As in all other reported cases of keratin 9 mutation in EPPK, this mutation lies within the highly conserved coil 1A of the rod domain, which is considered to play a role in the correct alignment of the coiled-coil molecules. ***Key words: keratin intermediate filament/helix initiation motif.*** ***J Invest Dermatol 109:113–115, 1997***

Epidermolytic palmoplantar keratoderma (EPPK) is a dominantly inherited autosomal disorder of keratinization characterized by diffuse thickening of the palms and soles and by epidermolytic hyperkeratosis histologically. For assembly of keratin intermediate filaments (KIF), type I keratins form two-chain molecules with type II keratins, and the two-chain molecules then align correctly each other (Steinert, 1990). Since keratin 9 (K9) belongs to the type I keratin, which is expressed exclusively in palms and soles, mutations of K9 have been examined in families suffering from this disease and were found in about half of the families (Bowden *et al*, 1994; Hennies *et al*, 1994; Reis *et al*, 1994; Torchard *et al*, 1994; Navsaria *et al*, 1995; Rothnagel *et al*, 1995; Kobayashi *et al*, 1996).[1,2] All these mutations of K9 lie within the coil 1A region of the rod domain, which is considered to play a role in the correct alignment of the coiled-coil molecules (Steinert *et al*, 1994). This was confirmed by an experiment that showed expression of a point-mutated K9 cDNA containing a G → A transition at nucleotide position 551 has a dominant negative effect on the assembly of KIF in cells (Kobayashi *et al*, 1996). We report herein a novel mutation of a highly conserved hydrophobic residue in the 1A domain of K9 in kindred of EPPK.

MATERIALS AND METHODS

An EPPK family was analyzed, whose pedigree is described in **Fig 1**. All affected family members (I.10 and II.1) presented diffuse yellowish hyperkeratosis limited strictly to the palms and soles. Patient II.1 presented the symptom during the first 6 mo after birth. On histologic examination, they showed typical features of epidermolytic hyperkeratosis, including many large keratohyalin granules and pronounced vacuolization of the cells in the upper stratum spinosum. No unaffected family members showed hyperkeratosis of the palms or soles.

Blood samples were obtained from 50 Japanese individuals who were not suffering from any congenital disorders and were processed for preparation of genomic DNAs. Genomic DNAs were obtained from the peripheral white blood cells as described previously (Poncz *et al*, 1982). To detect K9 mutations, highly conserved residues in the coil 1A of K9 were amplified by the polymerase chain reaction (PCR). Primer sequences are 5′-GGTATTCTGACTGCTAATGAG-3′ (sense) and 5′-CTTCTGGATAGCAGCAGGTCC-3′ (anti-sense). Approximately 0.3 μg of genomic DNA was amplified in each 100-μl PCR mixture consisting of 10 mM Tris(hydroxymethyl)aminomethane hydrochloride (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, all four deoxynucleotide triphosphate (each at 200 mM), each primer at 1 μM, and 5.0 units of *Taq* polymerase (Perkin-Elmer, Urayasu, Japan). PCR conditions were initial denaturation at 94°C for 2 min; 30 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min; and final extention at 72°C for 7 min. PCR products were subjected directly to automated nucleotide sequencing (Applied Biosystems, Foster City, CA).

The mutation was confirmed by the mismatch PCR method and allele-specific oligonucleotide (ASO) hybridization. For the mismatch PCR, the following probes were used: 5′-TGGAGGAGGCTATAGTAGTTC-3′ (sense) and 5′-AAGAGGCCAGCCGAGAGTTGA-3′ (anti-sense). PCR conditions were the same as above except for the annealing temperature, which was 48°C instead of 55°C. PCR products were digested by *Hin*cII,

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Abbreviations: EPPK, epidermolytic palmoplantar keratoderma; ASO, allele-specific oligonucleotide.

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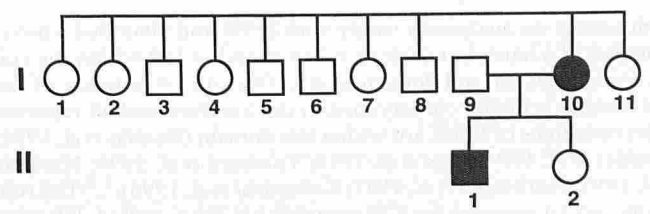

Figure 1. Pedigree of the family with EPPK. Solid symbols, affected individuals; open symbols, unaffected; ■, □, male; ●, ○, female.

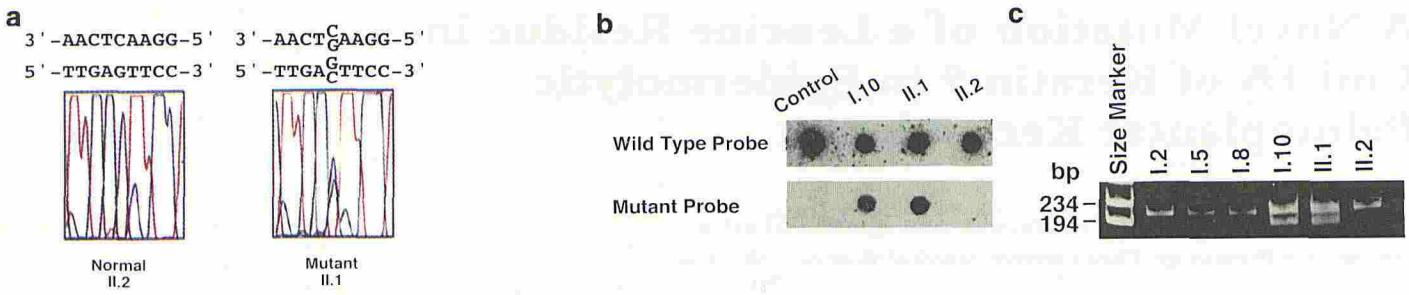

Figure 2. Identification and verification of the missense mutation L159V in one K9 allele in affected individuals. *(a)* Automated sequencing reveals a C → G transversion at nucleotide position 541, which converts a leucine residue (CTC) to a valine (GTC). The upper and the lower sequences are sense and anti-sense sequences, respectively. *(b)* ASO showed that signals for the wild-type and the mutant sequences are present in the PCR products of patients I.10 and II.1, but only the wild-type signal is present in the unaffected family member II.2 and the control sample. *(c)* For the mismatch PCR method, PCR was performed with a modified primer that creates a *Hinc*II recognition site only if codon 159 contains the mutant. After digestion of the PCR products with *Hinc*II, the fragments were analyzed on a 5% polyacrylamide gel. The fragments of 178 bp and 197 bp represent the digested PCR products from the mutant allele and the PCR products from the wild-type allele, respectively. Consequently, two fragments of 197 and 178 bp were observed in the PCR products of affected family members (I.10 and II.1), whereas the 197-bp fragment alone was observed in the PCR products of unaffected family members (I.2, I.5, I.8, and II.2).

and the digestion products were electrophoresed on 5% polyacrylamide gel. The fragments of 178 bp and 197 bp represent the digested PCR products from the mutant allele and the PCR products from the wild-type allele, respectively. The ASO hybridization was performed as described elsewhere (Dietz *et al*, 1992), and the probes were used as follows: wild type, 5′-ATGCAGGAACTCAATTCTC-3′; mutant type, 5′-ATGCAGGAAGTCAATTCTC-3′. The numbering of base pairs and amino acids is according to Langbein *et al* (1994).

RESULTS

The pedigree of the family with EPPK analyzed in this study is described in **Fig 1**. To detect K9 mutations, we amplified highly conserved residues in coil 1A of K9 by PCR. Direct nucleotide sequencing of the PCR products in all affected family members (I.10 and II.1) revealed a C → G transversion at nucleotide position 541 that converts a leucine residue (CTC) to a valine (GTC) in one allele (Langbein *et al*, 1994). This mutation, designated L159V, was not detected in an unaffected family member, II.2, by direct nucleotide sequencing. L159V was verified by the mismatch PCR method and ASO. Mismatch PCR was designed to create a *Hinc*II recognition site only if codon 159 contains the mutant. As shown in **Fig 2**, the PCR products from 50 control individuals and unaffected family members (I.2, I.5, I.8, and II.2) did not possess the *Hinc*II recognition site. Only the PCR products from the affected family members (I.10 and II.1) demonstrated the presence of this mutation. Assessment of the PCR products from control individuals and the unaffected family member (II.2) by ASO also failed to demonstrate the presence of this mutation **(Fig 2)**.

DISCUSSION

In this study we analyzed a family with EPPK and identified a novel mutation of a leucine residue to valine at codon 159 within the coil 1A segment of the rod domain of K9. The coil 1A segment of the rod domain is highly conservative in the keratins, and all reported point mutations of EPPK are within this domain (Bonifas *et al*, 1994; Hennies *et al*, 1994; Reis *et al*, 1994; Torchard *et al*, 1994; Navsaria *et al*, 1995; Rothnagel *et al*, 1995; Kobayashi *et al*, 1996).[1,2] The role of the coil 1A segment for KIF assembly has been studied. Filament assembly and cross-linking studies proposed a structural model for molecular interactions in KIF (Hatzfeld and Weber, 1992; Steinert *et al*, 1993a, 1993b; Compton, 1994). According to this model, the first 10 or 11 residues of the coil 1A segment of one molecule overlap with the last 10 or 11 residues of the coil 2B segment of the next molecule (Steinert, 1993). On the other hand, a filament disassembly study reported that residue positions 7–16 of the coil 1A segment are critical for the stability of KIF (Steinert *et al*, 1993c). The leucine residue at codon 159 of K9 is the seventh residue of the coil 1A segment that is considered to be important for the assembly of KIF anyway. In fact, the point mutation of this leucine residue to a phenylalanine residue of keratin 14 was reported in epidermolysis bullosa simplex (Yamanishi *et al*, 1994).

In our case, the L159V residue exchange appears undramatic, because leucine and valine are nonpolar residues and the substitution changes only the length of the hydrocarbon side chains. A point mutation of a nonpolar methionine residue to a nonpolar valine residue at codon 156 of K9, however, was also reported in EPPK (Hennies *et al*, 1994). In addition, it was reported that even more conservative substitutions could have profound deleterious effects on filament network formation and 10-nm filament assembly of vimentin. Substitution of glutamic acid for aspartic acid in the end of helix 2B of vimentin, which changed only the length of the hydrocarbons side chains, altered intermediate filament network formation *in vitro* (McCormick *et al*, 1993). Therefore, the L159V residue exchange will lead to the destabilization of filament network formation and 10-nm filament assembly of K9.

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